AD_____

Award Number: DAMD17-99-1-9271

TITLE: Novel Peptide/Protein Delivery System Targeting erbB2-Overexpressing Breast Cancer Cells

PRINCIPAL INVESTIGATOR: Dihua Yu, M.D., Ph.D.

CONTRACTING ORGANIZATION: The University of Texas
M. D. Anderson Cancer Center
Houston, Texas 77030

REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2002	Annual (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE
Novel Peptide/Protein Delivery System Targeting erbB2-Overexpressing Breast Cancer Cells

5. FUNDING NUMBERS
DAMD17-99-1-9271

6. AUTHOR(S)
Dihua Yu, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Texas
M. D. Anderson Cancer Center
Houston, Texas 77030
E-Mail:
Dyu@notes.mdacc.tmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The major goal of this study is to develop a penetratin-based delivery system **targeting specifically ErbB2-overexpressing breast cancer cells.** During this funding year, we focused on the delivery of erbB2 signal-blocking ESP peptides (objective 2). Because of the complexity of biotin-penetratin-AHNP-ESP, the synthesis was unsuccessful. We therefore began to try different approaches. We take Stat3 as an alternative target because we found that Stat3 activation is correlated with erbB2 expression levels, which constitutes a promising therapeutic target in treating erbB2-overexpressing breast cancers. We successfully synthesized Stat3-blocking peptide (Stat3BP), and further conjugated Stat3BP to NeutrAvidin. Through the interaction between NeutrAvidin and biotin, Stat3BP could be linked to biotin-penetratin-AHNP. Thus, We have developed a penetratin-based delivery system **targeting specifically ErbB2-overexpressing breast cancer cells**, and because of the modular nature of this system, we can link different peptides to it easily without synthesizing the penetratin-AHNP domain. Our effort has pointed a very positive direction for the next year, when we will test effects of various ErbB2 signal-blocking peptides using the ErbB2-targeting penetratin delivery system.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, penetratin, erbB2-targeting, peptide delivery, novel therapies	15
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

Introduction

The major goal of this study is to explore the possibility of developing a penetratin-based targeted delivery system to introduce specific therapeutic peptides into ErbB2-overexpressing breast cancer cells. In our work, we have been using the peptides derived from Tat sequence to develop a peptide/protein delivery system. The therapeutic peptides we choose are designed to block Shc binding and Stat3 activation as described below.

Some proteins, such as *Drosophila* homeoprotein *Antennapedia* and HIV Tat are able to translocate through the plasma membrane and move inside the cell nucleus to transactivate gene expression (Frankel and Pabo, 1988; Joliot et al., 1991). The peptide sequences that are responsible for such translocation were localized within a 16-amino acid domain of *Antennapedia* and a 13-amino acid domain of Tat, respectively (Derossi et al., 1994; Vives et al., 1997). When these peptide sequences (collectively named "penetratins") were linked to other peptides or proteins, they were able to carry the attached peptide or protein (up to 30kDa) into the cells when they were added to cell culture medium (Fawell et al., 1994). This unique property of penetratins can be utilized for delivery of therapeutic peptides/proteins (Bonfanti et al., 1997; Fahraeus et al., 1996). Penetratin-tagged peptides derived from the C-terminal domain of the tumor suppressor p53 restored the growth suppression function of p53 in cancer cells harboring a p53 mutation (Selivanova et al., 1997). Protein delivery by Tat penetratin was reported to be rapid and highly efficient, virtually all the cells received exogenous proteins within 1 hr (Fawell et al., 1994). Internalization of penetratin does not seem to rely on any specific cell surface receptor, since translocation of penetratins is endocytosis-independent and can occur at 4°C (Derossi et al., 1996). Furthermore, penetratin has translocated into every cell line tested so far (Bonfanti et al., 1997; Derossi et al., 1996; Derossi et al., 1994; Fahraeus et al., 1996; Fawell et al., 1994; Frankel and Pabo, 1988; Joliot et al., 1991; Mann and Frankel, 1991; Selivanova et al., 1997; Vives et al., 1997). Intraperitoneal injection of the 120kDa β-galactosidase protein, fused to Tat protein, results in delivery of the biologically active fusion protein to all tissues in mice (Schwarze et al., 1999). These data suggest that penetratin may be developed into a highly effective delivery system for therapeutic peptides or proteins and may have great applications in breast cancer therapy.

Overexpression of ErbB2 was found in ~ 30% of breast cancers and shown to correlate with the number of lymph node metastases and poor prognosis of the patients (Slamon et al., 1989; van de Vijver et al., 1988). *ErbB2* gene overexpression leads to enhanced metastatic potential and increased chemoresistance of breast cancer cells (Yu et al., 1998b; Yu et al., 1996), hence poor clinical outcome of patients (Yu and Hung, 1995). Therefore *ErbB2* should serve as an excellent target for the development of novel cancer therapies.

Tyrosine phosphorylated ErbB2 was known to bind to Shc (Segatto et al., 1993; Xie et al., 1995), which subsequently activates the ras/MAP (mitogen activated protein) kinase pathway. ErbB2-overexpressing 435.eB breast cancer cells have dramatically higher ERK activity than the ErbB2-lowexpressing parental MDA-MB-435 cells. Also, the activation of the Shc-->ERK pathway contributes to increased invasion/metastasis and Taxol-resistance in the ErbB2-overexpressing 435.eB breast cancer cells (Yu et al., 1996). We have been trying to interfere with the association between tyrosine phosphorylated p185 and Shc which may block the activation of the ErbB2-Shc downstream pathway in ErbB2-overexpressing breast cancer cells.

The signal transducers and activators of transcription factors (STATs) comprise a family of transcription factors that function as downstream effectors of cytokine and growth factor receptor signaling (Bromberg, 2000; Buettner et al., 2002). In response to growth factors or cytokines binding to their receptors, STATs become phosphorylated on tyrosine residue located near the C-terminus (Tyr705 in Stat3). Upon phosphorylation, STAT proteins form dimers, and then are translocated into the nucleus and bind to STAT specific DNA-response elements, thereby regulating gene expression (Bromberg, 2000; Buettner et al., 2002). Downstream targets of STATs include apoptosis inhibitors (Bcl-x_L), cell cycle regulators (cyclins D1/D2, c-Myc), and inducers of angiogenesis (VEGF) (Bromberg, 2000; Buettner et al., 2002). In normal cells, STAT-mediated gene regulation is tightly regulated. However, in certain types of cancer cells, STATs are constitutively activated and lead to aberrant cell growth, transformation, and progression (Niu et al., 2002). In

human cancer, there is a high frequency of activation of Stat1, Stat3, and Stat5, with a much higher incidence of abnormal Stat3 activation in almost all the tumors studied (Buettner et al., 2002). Use of dominant-negative and activated mutant Stat3, and antisense oligonucleotides, in relevant cell culture, animal models, and patient samples have provided a high degree of validation for Stat3 as a cancer therapeutic target (Bromberg et al., 1999; Catlett-Falcone et al., 1999; Epling-Burnette et al., 2001; Grandis et al., 2000; Nielsen et al., 1999). One report showed that Stat3 was constitutively activated (++ ~ +++ nuclear positive staining) in 50% of breast carcinomas as determined by immunohistochemistry (IHC) (Berclaz et al., 2001). Moreover, inhibition of constitutively active Stat3 by a JAK-Stat inhibitor and a dominant negative mutant of Stat3 suppressed the growth of human breast cancer cells *in vitro* (Burke et al., 2001). Hence, Stat3 is an excellent target for the development of novel therapeutics for breast cancers. We found that in multiple breast cancer cell lines, Stat3 activation is correlated with ErbB2 expression levels (see Figure 1). Therefore, we are also trying to block Stat3 dimerization/activation specifically in ErbB2-overexpressing breast cancer cells, which may effectively inhibit ErbB2-mediated malignant phenotypes.

Body

As shown in the previous annual report, we modified the order of the Objectives of the original proposal; i.e. we have pursued Objective 3 before pursuing Objective 2. We successfully developed an ErbB2-targeting delivery system by conjugating penetratin peptide to the ErbB2-binding peptide AHNP (Park et al., 2000) (penetratin-AHNP). We have achieved major goals proposed in Objective 1 (tasks 1, 2, 3) and Objective 3 (tasks 7, 8) of the original proposal. Our next step, which covers Objective 2 of the original proposal, is to use this system to deliver the potential ErbB2 signal-blocking peptides, and then test the effect on ErbB2 signaling. Therefore, this report will focus discussion on our effort in pursuing Objective 2 of the original proposal.

Objective 2: To test the effect of ErbB2 signal-blocking peptides using penetratin delivery (includes Tasks 4, 5, 6)

1: Synthesis of Shc-blocking phospho-peptides derived from ErbB2 C-terminal domain conjugated with penetratin-AHNP

1. The sequence of peptide ESP (ErbB2-derived Shc-binding peptide; AFENPEY(p)LTQ) is derived from the consensus sequence of the four Shc binding sites on ErbB2 cytoplasmic tail (Dankort et al., 1997). We tried to synthesize the ESP peptide conjugated to the delivery system (biotin-penetratin-AHNP-ESP). We have worked with several peptide companies; since it is not an easy synthesis, only one company is willing to try. Unfortunately, after more than six months of trying, the company was not able to synthesize biotin-penetratin-AHNP-ESP (see the attached emails). The failure of peptide synthesis could be due to the following reasons: a. the length of the peptide is too long (32 a.a. plus biotin); b. the incorporation of phosphorylated tyrosine residue and a disulfide bond in AHNP further complicated the synthesis. Dr. Martin Campbell in our institution's peptide synthesis Core Lab is now trying to synthesize small stretches of peptides (about 20 amino acids each) and then link the peptides together. To save time, we also began another alternative approach (described below) to circumvent this problem.
2. Signal transducers and activators of transcription (STATs) are transcription factors activated in response to cytokines and growth factors. Constitutively active Stat3 has been shown to mediate oncogenic transformation in cultured cells and induce tumor formation in mice, and inhibition of the Stat3 signaling pathway significantly suppresses the growth of ovarian and breast cancer cell lines harboring constitutively active Stat3 (Burke et al., 2001). Interestingly, we found that in multiple breast cancer cell lines, Stat3 activation is correlated with ErbB2 expression levels (Figure 1). Our lab also has found that Stat3 contribute to

ErbB2-mediated chemoresistance by transcriptionally upregulating p21 (Yu et al., 1998a). Therefore, we are also focusing on the inhibition of Stat3 signaling as a potential therapeutic approach for treating ErbB2-overexpressing breast cancers.

On the other hand, we are trying to build our peptides by combining the smaller peptide "domains" using a similar approach as the biotin/streptavidin system in the previous literature (Schwarzenberger et al., 1996). We have successfully synthesized the Stat3-blocking peptide (Stat3BP), which inhibits the dimerization therefore the activation of Stat3 (Table 1.). Then Stat3BP was conjugate through the N-terminal cysteine residue to maleimide activated NeutrAvidin, a biotin-binding peptide (Pierce, Rockford, IL), to obtain Stat3BP-NeutrAvidin. To confirm that the conjugation is successful, the conjugation product (supposedly Stat3BP-NeutrAvidin) was separated by SDS-PAGE and subjected to immunoblotting with anti-phosphotyrosine antibody (PY20, BD Transduction Lab.). As shown in Figure 2, there are major bands with the sizes ranging from around 30 to 60 kDa. Since there is no phosphorylated tyrosine residue on NeutrAvidin, and the size of Stat3BP is only 1.3 kDa, the phosphotyrosine signals must come from the Stat3BP conjugated to NeutrAvidin (molecular weight: ~60 kDa). We can then mix biotin-penetratin-AHNP and antiStat3-NeutrAvidin. Through the high affinity between biotin and NeutrAvidin, Stat3BP can be linked with the ErbB2-targeting penetratin-AHNP. We are now testing if Stat3BP is successfully translocated into ErbB2-overexpressing cancer cells by penetratin-AHNP.

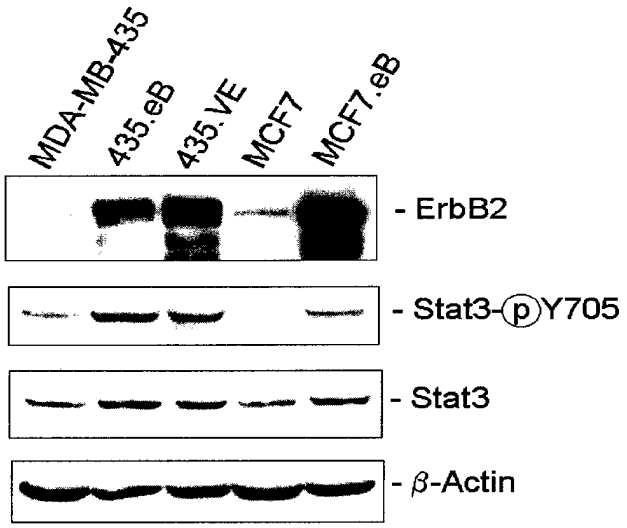

Figure 1. ErbB2 protein levels and Stat3 activation

Lysates from breast cancer cell lines MDA-MB-435, MCF7, and their ErbB2 transfectants 435.eB (wild-type), 435.VE (activated), and MCF7.eB (wild-type) were immunoblotted with the antibodies indicated.

Table 1. The inhibition of Stat3 DNA binding by phosphopeptides

Peptide	Sequence	DB_{50} (μM)[a]	
Stat3BP	Ac Y(p)KPQTV amide	0.15	
Stat3 SH2-binding peptides	PY(p)LKTK	235±36	(Turkson et al., 2001)
	PY(p)L	182±15	(Turkson et al., 2001)
	AY(p)L	217±55	(Turkson et al., 2001)

[a] DB_{50}, concentration of peptide at which DNA binding activity is reduced by 50%

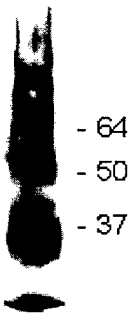

Figure 2. Conjugation of Stat3BP and maleimide activated NeutrAvidin biotin-binding protein

Following the protocol provided by the manufacturer, Stat3BP was incubated in conjugation solution with NeutrAvidin. The product was then dialyzed to eliminate non-conjugated Stat3BP. This final product was immunoblotted with anti-phosphotyrosine antibody.

Key Research Accomplishments

1. We found that Stat3 activation is correlated with ErbB2 expression levels, which constitutes a promising therapeutic target in treating ErbB2-overexpressing breast cancers.
2. We successfully synthesized Stat3-blocking peptide (Stat3BP), and further conjugated Stat3BP to NeutrAvidin. Through the interaction between NeutrAvidin and biotin, Stat3BP could be linked to biotin-P3 (TAT)-AHNP.

Reportable Outcomes:

-Manuscripts, abstracts, presentations
We are not to the stage of writing manuscript yet.

-Patents and licenses applied for and/or issued
We intend to patent our findings in the future.

-Degrees obtained that are supported by this award	None
-Development of cell lines, tissue or serum repositories	None
-Informatics such as databases and animal models	None
-Funding applied for based on work supported by this	None

Conclusions

The major goal of this work is to develop a penetratin-based delivery system targeting specifically ErbB2-overexpressing breast cancer cells. During this funding year, we focused on the delivery of erbB2 signal-blocking ESP peptides (objective 2). Because of the complexity of biotin-penetratin-AHNP-ESP, the synthesis was unsuccessful. We therefore began to try different approaches. We take Stat3 as an alternative target because we found that Stat3 activation is correlated with erbB2 expression levels, which constitutes a promising therapeutic target in treating erbB2-overexpressing breast cancers. We successfully synthesized Stat3-blocking peptide (Stat3BP), and further conjugated Stat3BP to NeutrAvidin. Through the interaction between NeutrAvidin and biotin, Stat3BP could be linked to biotin-penetratin-AHNP.

In this way, we have developed a penetratin-based delivery system targeting specifically ErbB2-overexpressing breast cancer cells, and because of the modular nature of this system, we can link different peptides to it easily without synthesizing the penetratin-AHNP domain. Our effort has pointed a very positive direction for the next year, when we will test effects of various ErbB2 signal-blocking peptides using the ErbB2-targeting penetratin delivery system.

We are very appreciative that the US AMRMC granted us one year of non-funded extension. We hope this will allow us to fulfill all the proposed tasks next year.

References

Berclaz, G., Altermatt, H. J., Rohrbach, V., Siragusa, A., Dreher, E., and Smith, P. D. (2001). EGFR dependent expression of STAT3 (but not STAT1) in breast cancer. International Journal of Oncology *19*, 1155-1160.

Bonfanti, M., Taverna, S., Salmona, M., D'Incalci, M., and Broggini, M. (1997). $p21^{WAF1}$-derived peptides linked to an internalization peptide inhibit human cancer cell growth. Cancer Res *57*, 1442-1446.

Bromberg, J. (2000). Signal transducers and activators of transcription as regulators of growth, apoptosis and breast development. Breast Cancer Research *2*, 86-90.

Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C., and Darnell, J. E., Jr. (1999). Stat3 as an oncogene. [erratum appears in Cell 1999 Oct 15;99(2):239.]. Cell *98*, 295-303.

Buettner, R., Mora, L. B., and Jove, R. (2002). Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clinical Cancer Research *8*, 945-954.

Burke, W. M., Jin, X., Lin, H. J., Huang, M., Liu, R., Reynolds, R. K., and Lin, J. (2001). Inhibition of constitutively active Stat3 suppresses growth of human ovarian and breast cancer cells. Oncogene *20*, 7925-7934.

Catlett-Falcone, R., Landowski, T. H., Oshiro, M. M., Turkson, J., Levitzki, A., Savino, R., Ciliberto, G., Moscinski, L., Fernandez-Luna, J. L., Nunez, G., *et al.* (1999). Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity *10*, 105-115.

Dankort, D. L., Wang, Z., Blackmore, V., Moran, M. F., and Muller, W. J. (1997). Distinct tyrosine autophosphorylation sites negatively and positively modulate neu-mediated transformation. Mol Cell Biol *17*, 5410-5425.

Derossi, D., Calvet, S., Trembleau, A., Brunissen, A., Chassaing, G., and Prochiantz, A. (1996). Cell internalization of the third helix of the antennapedia homeodomain is receptor-independent. J Biol Chem *271*, 18188-18193.

Derossi, D., Joliot, A. H., Chassaing, G., and Prochiantz, A. (1994). The third helix of the antennapedia homeodomain translocates through biological membranes. J Biol Chem *269*, 10444-10450.
Epling-Burnette, P. K., Liu, J. H., Catlett-Falcone, R., Turkson, J., Oshiro, M., Kothapalli, R., Li, Y., Wang, J. M., Yang-Yen, H. F., Karras, J., *et al.* (2001). Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. Journal of Clinical Investigation *107*, 351-362.
Fahraeus, R., Paramio, J. M., Ball, K. L., Lain, S., and Lane, D. P. (1996). Inhibition of pRb phosphorylation and cell-cycle progression by a 20-residue peptide derived from $p16^{CDKN2/INK4A}$. Curr Biol *6*, 84-91.
Fawell, S., Seery, J., Daikh, Y., Moore, C., Chen, L. L., Pepinsky, B., and Barsoum, J. (1994). Tat-mediated delivery of heterologous proteins into cells. Proc Natl Acad Sci USA *91*, 664-668.
Frankel, A. D., and Pabo, C. O. (1988). Cellular uptake of the tat protein from human immunodeficiency virus. Cell *55*, 1189-1193.
Garcia, R., Bowman, T. L., Niu, G., Yu, H., Minton, S., Muro-Cacho, C. A., Cox, C. E., Falcone, R., Fairclough, R., Parsons, S., *et al.* (2001). Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene *20*, 2499-2513.
Grandis, J. R., Drenning, S. D., Zeng, Q., Watkins, S. C., Melhem, M. F., Endo, S., Johnson, D. E., Huang, L., He, Y., and Kim, J. D. (2000). Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. Proceedings of the National Academy of Sciences of the United States of America *97*, 4227-4232.
Joliot, A., Pernelle, C., Deagostini-Bazin, H., and Prochiantz, A. (1991). Antennapedia homeobox peptide regulates neural morphogenesis. Proc Nat Acad Sci USA *88*, 1864-1868.
Mann, D. A., and Frankel, A. D. (1991). Endocytosis and targeting of exogenous HIV-1 tat protein. EMBO J *10*, 1733-1739.
Nielsen, M., Kaestel, C. G., Eriksen, K. W., Woetmann, A., Stokkedal, T., Kaltoft, K., Geisler, C., Ropke, C., and Odum, N. (1999). Inhibition of constitutively activated Stat3 correlates with altered Bcl-2/Bax expression and induction of apoptosis in mycosis fungoides tumor cells. Leukemia *13*, 735-738.
Niu, G., Wright, K. L., Huang, M., Song, L., Haura, E., Turkson, J., Zhang, S., Wang, T., Sinibaldi, D., Coppola, D., *et al.* (2002). Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene *21*, 2000-2008.
Page, C., Huang, M., Jin, X., Cho, K., Lilja, J., Reynolds, R. K., and Lin, J. (2000). Elevated phosphorylation of AKT and Stat3 in prostate, breast, and cervical cancer cells. International Journal of Oncology *17*, 23-28.
Park, B. W., Berezov, A., Wu, C. W., Zhang, X., Dua, R., Zhang, H. T., Wang, Q., Kao, G., O'Rourke, D. M., Green, M. I., and Murali, R. (2000). Rationally designed anti-HER2/neu peptide mimetic disables $p185^{HER2/neu}$ tyrosine kinases in vitro and in vivo. Nat Biotech *18*, 194-198.
Schwarze, S. R., Ho, A., Vocero-Akbani, A., and Dowdy, S. F. (1999). In vivo protein transduction: delivery of a biologically active protein into the mouse.
Schwarzenberger, P., Spence, S. E., Gooya, J. M., Michiel, D., Curiel, D. T., Ruscetti, F. W., and Keller, J. R. (1996). Targeted gene transfer to human hematopoietic progenitor cell lines through the c-kit receptor. Blood *87*, 472-478.
Segatto, O., Pelicci, G., Giuli, S., Digiesi, G., Di Fiore, P. P., McGlade, J., Pawson, T., and Pelicci, P. G. (1993). Shc products are substrates of erbB-2 kinase. Oncogene *8*, 2105-2112.
Selivanova, G., Iotsova, V., Okan, I., Fritsche, M., Strom, M., Groner, B., Grafstrom, R. C., and Wiman, K. G. (1997). Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nature Medicine *3*, 632-638.

Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A., and McGuire, W. L. (1989). Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science (Washington, DC) *244*, 707-712.

Turkson, J., Ryan, D., Kim, J. S., Zhang, Y., Chen, Z., Haura, E., Laudano, A., Sebti, S., Hamilton, A. D., and Jove, R. (2001). Phosphotyrosyl peptides block Stat3-mediated DNA binding activity, gene regulation, and cell transformation. Journal of Biological Chemistry *276*, 45443-45455.

van de Vijver, M. J., Peterse, J. L., Mooi, W. J., Wisman, P., Lomans, J., Dalesio, O., and Nusse, R. (1988). *Neu*-protein overexpression in breast cancer: association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. N Engl J Med *319*, 1239-1245.

Vives, E., Brodin, P., and Lebleu, B. (1997). A truncated HIV-1 tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J Biol Chem *272*, 16010-16017.

Xie, J., LeBaron, M. J., Nevalainen, M. T., and Rui, H. (2002). Role of tyrosine kinase Jak2 in prolactin-induced differentiation and growth of mammary epithelial cells. Journal of Biological Chemistry *277*, 14020-14030.

Xie, Y., Li, K., and Hung, M.-C. (1995). Tyrosine phosphorylation of Shc proteins and formation of Shc/Grb2 complex correlate to the transformation of NIH3T3 cells mediated by the point-mutation activated *neu*. Oncogene *10*, 2409-2413.

Yu, D., and Hung, M.-C. (1995). HER-2/neu gene in human cancers. In Molecular Basis of Oncology, E. Freireich, and S. A. Stass, eds. (Cambridge, Blackwell Scientific Publications Inc.), pp. 131-162.

Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T. J., and Hung, M.-C. (1998a). Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of $p21^{Cip1}$, which inhibits $p34^{Cdc2}$ kinase. Molecular Cell *2*, 581-591.

Yu, D., Liu, B., Sun, D., Jing, T., Price, J. E., Singletary, S. E., Hortobagyi, G. N., and Hung, M.-C. (1998b). Overexpression of both $p185^{c\text{-}erbB2}$ and $p170^{mdr\text{-}1}$ renders breast cancer cells highly resistant to Taxol. Oncogene *16*, 2087-2094.

Yu, D., Liu, B., Tan, M., Li, J., Wang, S.-S., and Hung, M.-C. (1996). Overexpression of c-erbB-2/neu in breast cancer cells confers increased resistance to Taxol via mdr-1-independent mechanisms. Oncogene *13*, 1359-1365.